Analytica Chimica Acta 830 (2014) 42–48

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Gold nanocatalyst-based immunosensing strategy accompanying catalytic reduction of 4-nitrophenol for sensitive monitoring of chloramphenicol residue

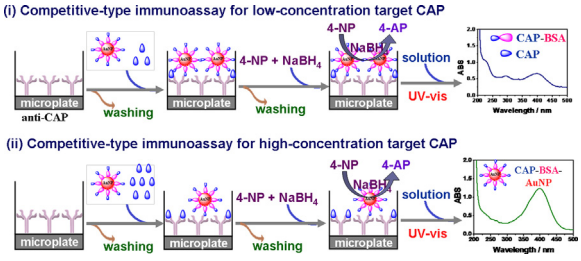
Xiaohua Que [a,c], Dianyong Tang [a], Biyun Xia [a], Minghua Lu [b,**], Dianping Tang [a,c,*]

[a] Chongqing Key Laboratory of Environmental Materials & Remediation Technologies, College of Materials and Chemical Engineering, Chongqing University of Arts and Sciences, Chongqing 402160, PR China
[b] Institute of Environmental and Analytical Science, School of Chemistry and Chemical Engineering, Henan University, Kaifeng, Henan 475004, PR China
[c] Key Laboratory of Analysis and Detection for Food Safety (Ministry of Education & Fujian Province), Department of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350108, PR China

HIGHLIGHTS

- We report a new competitive-type immunoassay protocol for the detection of antibiotic residue.
- Gold nanoparticles are used as nanotags and nanocatalysts for signal amplification.
- The assay is implemented by catalytic reduction of gold nanocatalysts toward 4-nitropheneol.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 March 2014
Received in revised form 21 April 2014
Accepted 25 April 2014
Available online 28 April 2014

Keywords:
Chloramphenicol
Foodstuff
Gold nanocatalyst
Immunoassay
Ultraviolet–visible spectroscopy

ABSTRACT

A new competitive-type immunosensing system based on gold nanoparticles toward catalytic reduction of 4-nitrophenol (4-NP) was developed for sensitive monitoring of antibiotic residue (chloramphenicol, CAP, used in this case) by using ultraviolet–visible (UV–vis) spectrometry. Gold nanoparticle (AuNP) with 16 nm in diameter was initially synthesized and functionalized with CAP–bovine serum albumin (CAP–BSA) conjugate, which were used as the competitor on monoclonal anti-CAP antibody-coated polystyrene microtiter plate (MTP). In the presence of target CAP, the labeled CAP–BSA on the AuNP competed with target CAP for the immobilized antibody on the MTP. The conjugated amount of CAP–BSA–AuNP on the MTP decreased with the increase of target CAP in the sample. Upon addition of 4-NP and $NaBH_4$ into the MTP, the carried AuNP could catalytically reduce 4-NP to 4-aminophenol (4-AP), and the as-produced 4-AP could be monitored by using UV–vis absorption spectroscopy. Experimental results indicated that the absorbance at 403 nm increased with the increment of target CAP concentration in the sample, and exhibited a dynamic range from 0.1 to 100 ng mL^{-1} with a detection limit (LOD) of 0.03 ng mL^{-1} at the $3s_{blank}$ level. Intra- and inter-assay coefficients of variation were lower than 5.5% and 8.0%, respectively. In addition, the methodology was evaluated for CAP spiked honey and milk samples, respectively. The recovery was 92–112%.

* Corresponding author at: Institution of Analytical Chemistry, College of Chemistry, Fuzhou University, Fuzhou 350108, PR China. Tel.: +86 591 2286 6125; fax: +86 591 2286 6135.
** Corresponding author. Tel.: +86 378 3881 589; fax: +86 378 3881 599.
E-mail addresses: minghua.lu2009@hotmail.com (M. Lu), dianping.tang@fzu.edu.cn (D. Tang).

http://dx.doi.org/10.1016/j.aca.2014.04.051
0003-2670/

1. Introduction

With regard to the presence of drug residues in the food chain, the ability to both rapidly and stably detect trace amount of small molecules (<1000 Da) is very important in the area of food safety. The existing analytical methods for small molecules include microbiological inhibition tests, immunoassays and chemical–physical methods [1]. By and large, all the single methods available have their characteristic advantages and disadvantages: microbiological tests are relatively low-cost but complex and slow, whereas immunoassays are highly sensitive and selective but expensive [2,3]. Chemical–physical methods based on high-priced instrumentation can quantitatively detect, however, the operations are complicated with many reagents and multiple steps involved [4]. An alternative method and strategy by combining the merits of immunoassay with quantitative chemical–physical method (e.g., UV–vis absorbance spectroscopy) would be advantageous.

Methods based on immunosensors and immunoassays with various signal generation principles have been developed for the detection of biomolecules in clinical diagnosis, food safety, and environmental protection [5–7]. Owing to the limitation of antigenic epitope on the small biomolecules, competitive-type immunoassay systems are usually utilized for the determination of small biomolecules [8]. Compared with sandwich-type immunoassay format, competitive-type immunoassay is simpler and can reduce the incubation time. However, it often suffers from a narrower linear range. Therefore, high-affinity antibody and appropriate label are usually employed for the amplification of detectable signal [9,10]. Recent research has looked to develop innovative and powerful nanoparticle labels, controlling and tailoring their properties in a very predictable manner to meet the requirements of specific applications.

A gold nanoparticle label is an ideal one in biotechnological systems due to its inherent advantages, such as easy preparation, good biocompatibility, and so on [11]. As far back as 1970s, colloidal gold particles were used as an immune-staining and contrast agent for electron microscopy [12]. Nowadays, gold nanoparticles have been extensively employed as the labels for different biological receptors, e.g., enzyme, DNA, antigen/antibody and other biomolecules [13,14]. More significantly, gold nanoparticles can be also used as catalysts in a number of chemical reactions. Yang [15] and Tang [16] designed two types of ultrasensitive electrochemical immunoassay using nanometer gold labels as catalysts. The catalytic properties mainly derived from the catalytic reduction of 4-nitrophenol by gold nanolabels. The Zhang group developed a rapid method for detection of aflatoxin M_1 by coupling superparamagnetic beads with gold labels [17]. Based on the characteristic of surface plasmon resonance absorption of gold nanoparticles, Zhu et al. constructed an optical sensor for detection of antibiotics by using UV–vis absorbance spectrometry [18]. The resonance wavelength usually depended on the nanoparticle's size, shape and local dielectric environment. In this case, the detectable signal was relatively weak. To improve this issue, our motivation in this study is to combine with the catalytic properties of gold nanoparticles to produce another new product with strong plasmon resonance efficiency, thus achieving the amplification of detectable signal.

Chloramphenicol (CAP), a bacteriostatic antimicrobial, is usually considered as an effective broad-spectrum antibiotic used in all major food-producing animals [19]. However, research has shown that chloramphenicol is a suspected carcinogen, even low doses of administered chloramphenicol may result in residues in edible tissues, and it can lead to a plastic anemia and dose-related reversible bone marrow depression in humans [20,21]. So, sensitive detection of chloramphenicol residues in foodstuff is very important. Herein, we design a new competitive-type immunoassay system for the detection of chloramphenicol (as a model) by coupling with catalytic properties of gold nanolabel toward 4-nitriphenol for signal amplification. The generated 4-aminophenol can be quantitatively measured by using UV–vis absorption spectroscopy, and the absorbance is indirectly proportional to the concentration of target CAP. The aim of this work is to exploit a newly hyphenated technique for determination of chloramphenicol residue in the analysis of food safety.

2. Experimental

2.1. Materials

Monoclonal Rabbit anti-chloramphenicol antibody (anti-CAP, 1.0 mg mL^{-1}) was purchased from Beijing Biosynth. Biotechnol. Co., Ltd., (Beijing, China). Chloramphenicol standards (CAP) with various concentrations were purchased from Beijing Dingguo Biotechnol. Co., Ltd., (Beijing, China). Bovine serum albumin (BSA, 96–99%) was purchased from Shanghai Medpep Co., Ltd., (Shanghai, China). $HAuCl_4$, 4-nitrophenol (4-NP), and $NaBH_4$ were purchased from Sinopharm Chem. Re. Co., Ltd., (Shanghai, China). 1-Ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma (Shanghai, China). Gold colloids (AuNP) with 16 nm in diameter were synthesized according to our previous report [22]. All reagents were of analytical grade and used as received without further purification.

A coating buffer (pH 9.6) consisted of 1.59 g Na_2CO_3, 2.93 g $NaHCO_3$ and 0.2 g NaN_3 in 1000 mL distilled water. A pH 7.4 phosphate-buffered saline (PBS, 0.01 M) (2.9 g $Na_2HPO_4{\cdot}12H_2O$, 0.24 g KH_2PO_4, 0.2 g KCl and 8.0 g NaCl) was prepared by adding the corresponding chemicals into 1000 mL distilled water. The blocking and washing buffers (PBST) were obtained by adding 1.0% BSA (w/v) and 0.05% Tween 20 (v/v), respectively.

2.2. Apparatus

Ultraviolet–vis absorption (UV–vis) spectra were recorded with an 1102 UV–vis spectrophotometer (Techcomp, China). Ultrapure deionized water was generated using a Millipore Milli-Q plus system. All pH measurements were made with a pH meter (MP 230, Mettler-Toledo Co., Switzerland) and a digital ion analyzer (Model PHS-3C, Dazhong Instruments, Shanghai, China).

2.3. Procedures

2.3.1. Preparation of BSA–CAP conjugates and their labeling with gold nanoparticles

BSA–CAP conjugates were prepared through the classical carbodiimide coupling [23,24]. Briefly, 60 mg of CAP was initially dissolved in 1 mL absolute ethanol, then the mixture was adjusted to pH 1 by using 0.1 M HCl, and then 80 mg zinc powder was added in the mixture to react for 40 min at 65 °C until the yellowish-brown clear liquid of reductive CAP was obtained. Following that, the reductive CAP was dissolved in borate buffer (0.2 M, pH 8.5) to 10 mg mL^{-1}. EDC (0.4 M) and NHS (0.1 M) were simultaneously added into the mixture, and incubated for 10 min at room temperature (RT) without agitation. Afterwards, BSA borate buffer solution (molar ratio of BSA and CAP was 1:100) was added dropwise to the solution. The resultant mixture was incubated for another 2 h at RT, desalted by dialyzing against 1000 volumes of PBS (0.01 M, pH 7.4) overnight, and stored at 4 °C for further use.

Next, the as-prepared BSA–CAP was utilized for the labeling of gold nanoparticles as follows [17]. Briefly, 1.0 mL of gold colloids ($C_{[AuNP]} \approx 24$ nM) was initially adjusted to pH 8–9 using 0.01 M NaOH, and 200 μL of 0.5 mg mL^{-1} BSA–CAP was then